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ATTN: MCMR-UV-CC
3100 Ricketts Point Road
Aberdeen Proving Ground, MD
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ATTN: MCMR-UV-RC
3100 Ricketts Point Road
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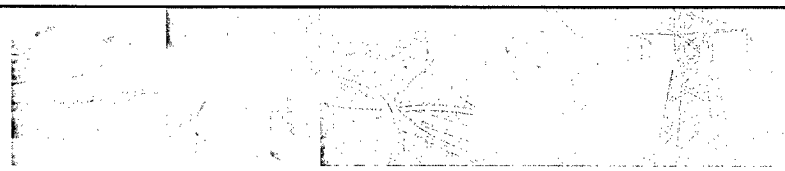

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Effects of Three Anesthetic Regimens on Bioengineering Methods Conducted on Ventral Abdominal Skin of Weanling Swine#

John S. Graham,[1,*] Frances M. Reid,[2] Nancy A. Niemuth,[2] Shawn M. Shumaker,[2] and Jack D. Waugh[2]

[1]Comparative Pathology Branch, Comparative Medicine Division, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland, USA
[2]Medical Research and Evaluation Facility, Battelle, Columbus, Ohio, USA

ABSTRACT

Background/Purpose: A weanling swine model was previously developed to study healing of cutaneous injuries induced by the blistering chemical warfare agent sulfur mustard. Noninvasive bioengineering methods are used in the model to monitor the progress of wound healing and evaluate the efficacy of treatments. It is necessary to anesthetize the animals to facilitate bioengineering data collection from ventral

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*Correspondence: John S. Graham, Ph.D., Comparative Pathology Branch, Comparative Medicine Division, U.S. Army Medical Research Institute of Chemical Defense, 3100 Ricketts Point Rd., Aberdeen Proving Ground, MD 21010-5400, USA; Fax: (410) 436-1132; E-mail: john.graham@amedd.army.mil.

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abdominal wound sites. As anesthetic agents affect cardiac output and deep vascular and cutaneous microcirculation in many species, there was a need to identify an anesthetic regimen with minimal effects on baseline measurements. The effects of three anesthetic regimens on reflectance colorimetry, transepidermal water loss, and laser Doppler perfusion imaging were studied in unexposed control animals. *Methods:* The following regimens were tested on six female Yorkshire swine (weanlings, 8–11 kg): repeated, separate intramuscular (i.m.) injections of ketamine HCl and xylazine HCl (K/X, at 20 ± 2 mg/kg and 2 ± 0.2 mg/kg, respectively), repeated i.m. injections of a tiletamine HCl/zolazepam HCl/xylazine HCl combination (T/X, at 2.2 mg/kg, 2.2 mg/kg, and 4.4 mg/kg, respectively), and the tiletamine HCl/zolazepam HCl/xylazine HCl combination as a preanesthetic and isoflurane inhalation to maintain anesthesia (T/X/Iso; dosage of tiletamine HCl/zolazepam HCl/xylazine HCl was the same as listed above, with 2.5–3.0% isoflurane in oxygen at an initial flow rate of 2 L/min, reduced to 1.0%–1.5% at 0.8–1.0 L/min for maintenance). Each regimen was administered in three iterations within a week (every other day), with a minimum 1-week washout period between regimens. *Results:* The effect of the anesthetic regimens on bioengineering assessments of ventral abdominal skin was evaluated. For reflectance colorimetry, regimens T/X and K/X had a narrower range of readings over the three testing days than T/X/Iso. Either T/X or K/X was suitable, with T/X preferred because of a lesser blanching effect. T/X or T/X/Iso were preferred for transepidermal water loss readings, because overall they depressed transepidermal water loss rates less than did K/X. T/X, T/X/Iso, and K/X all affected cutaneous blood flow, with no clear preference. *Conclusions:* Overall, T/X produced the most consistent results with the fewest anesthetic effects.

Key Words: Reflectance colorimetry; Transepidermal water loss; Laser Doppler perfusion imaging; Anesthesia; Swine.

INTRODUCTION

We recently developed a weanling swine model to examine healing of cutaneous injuries induced by the blistering chemical warfare agent sulfur mustard [SM] (1,2). Because of the similarities between human and porcine skin (3–9), swine were chosen for the model. Noninvasive bioengineering methods are used in the model to monitor the progress of wound healing and evaluate the efficacy of treatments (10).

In our wound healing studies, SM is applied to ventral abdominal skin, shown in weanling pigs to be more sensitive to SM than skin on the dorsum (1,11). In addition, the ventral abdominal skin on these animals is more pliable and lower in pelage density than that found on the dorsum, making it easier to manipulate and keep free of hair stubble during treatments, clinical observations, and bioengineering measurements. For these reasons, we found it practical to use ventral abdominal skin in our wound-healing studies.

Although swine are amenable to habituation and can be trained to allow noninvasive biophysical skin measurements to be obtained from experimental sites on the dorsum without the need for restraint or anesthesia (12), it is very difficult to get these animals to remain calm enough to allow such measurements on ventral abdominal skin. Excessive movement makes collection of valid bioengineering data very difficult.

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In addition, experimental sites on the ventral abdominal surface are most easily examined with the animal in dorsal recumbency. The excessive restraint that would be required to keep the animals relatively motionless in this position would likely exert undue emotional stress on the animals that could, in turn, affect release of vasoactive amines, cutaneous blood flow, biophysical measurements, wound healing, and the overall health of the animals. In humans, it is generally accepted that for optimal wound healing to occur, a healthy immunological (13), nutritional/metabolic (14), and psychological (15) status needs to be maintained. Optimal wound healing of cutaneous injuries in swine likely require these same conditions. Therefore it is necessary to anesthetize our animals to facilitate bioengineering data collection from ventral abdominal wound sites under stress-free conditions.

Anesthetic agents affect cardiac output and deep vascular and cutaneous microcirculation in many species, including swine (16–18). These effects may interfere with the evaluation of skin lesions. Skin color, epidermal barrier function, cutaneous blood flow, and the mechanical properties of hardness and elasticity, among other parameters, are used in efficacy evaluations of treatment regimens in our SM wound-healing studies (10). To identify an anesthetic regimen with minimal effects on bioengineering assessments of ventral abdominal skin in swine, a study was conducted to examine the effects of various anesthetic regimens on reflectance colorimetry (RC), evaporimetry [transepidermal water loss (TEWL)], and laser Doppler perfusion imaging (LDPI) in a weanling swine model (1).

MATERIALS AND METHODS

Animals

Six female Yorkshire crossbred swine (5- to 7-week-old weanlings, *Sus scrofa*), weighing 8–11 kg were quarantined for 7 days and screened for evidence of disease before use. Specific pathogen-free (SPF) animals were purchased from Isler Genetics, Inc. of Prospect, OH. The SPF swine were proven free of pseudorabies, porcine reproductive and respiratory syndrome, transmissible gastroenteritis, *Actinobacillus pleuropneumonia*, enzootic pneumonia, *Streptococcus suis*, *Tremponema hyodysenteria*, *Pasturella multocida*, ectoparasites, and endoparasites by quarterly health inspections that included necropsies. The swine were maintained under an animal care and use program accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). The Institutional Animal Care and Use Committee at Battelle, Columbus, OH, approved the experimental protocol. An Edstrom automatic watering system provided *ad libitum* potable water. Swine were initially fed 0.3 kg of a laboratory swine grower ration (PMI Feeds, Inc., St. Louis, MO) twice a day, with the amount gradually increased over 4 weeks to 0.5 kg twice a day. Animals were housed individually in stainless steel mobile swine enclosures (Britz–Heidbrink, Inc., Wheatland, WY). The animal rooms were maintained between 10 and 27°C, with 50% ± 20% relative humidity, using a minimum of 10 complete air changes per hour of 100% conditioned fresh air. Animal rooms were maintained on a 12-hour light/dark, full-spectrum lighting cycle with no twilight.

Procedures

The weanling swine model developed to assess SM burn healing was used (1), except for the following: a chemical depilatory compound (Magic®; Carson Products, Co., Savannah, GA) was used to remove hair stubble the day before initial testing began for each animal. Thereafter, electric clippers were used to gently remove regrowth, as needed. Following induction of anesthesia, animals were placed in dorsal recumbency on a therapeutic heating pad on an examination table equipped with positioning pillows. Six experimental sites (5 cm × 5 cm) were demarcated on the ventral abdominal surface, three sites per side parallel to and approximately 2.5 cm lateral to the teat line and located between the axillary and inguinal areas. A plastic template was used for even spacing and consistent anatomical positioning of sites among animals. No wounds were generated in this study, because SM was not applied to the sites. Noninvasive bioengineering methods were thus conducted on normal skin, yielding baseline measurements.

The following three anesthetic regimens were tested:

1. K/X = Repeated, separate intramuscular (i.m.) injections of 20 ± 2 mg/kg body weight of a 100 mg/mL ketamine HCl solution (Ketaset®; Fort Dodge, IA) and 2 ± 0.2 mg/kg of a 100 mg/mL xylazine HCl solution (Ben Venue Laboratories, Inc., Bedford, OH).
2. T/X = tiletamine HCl/zolazepam HCl/xylazine HCl combination. A 5-mL bottle of Telazol® (250 mg of tiletamine HCl and 250 mg of zolazepam HCl, Elkins–Sim, Inc., Cherry Hill, NJ) was reconstituted with 5 mL of xylazine HCl (100 mg/mL). The combination was administered in repeated i.m. injections with the following dosages: 2.2 mg/kg body weight of tiletamine HCl, 2.2 mg/kg of zolazepam HCl, and 4.4 mg/kg of xylazine HCl.
3. T/X/Iso = tiletamine HCl/zolazepam HCl/xylazine HCl combination as a preanesthetic for intubation and inhalation of isoflurane (Solvay Animal Health Inc., Mendeta Heights, MN) to maintain anesthesia. The dosages for tiletamine HCl, zolazepam HCl, and xylazine HCl were as described above. Anesthesia was initiated by using a concentration of 2.5–3.0% isoflurane in oxygen at an initial flow rate of 2 L/min using a CDS2000 Anesco anesthetic machine with an Ohio® Calibrated Isoflurane Vaporizer (Anesco, Inc., Georgetown, KY). The isoflurane concentration was reduced to 1.0–1.5%, and oxygen flow rate reduced to 0.8–1.0 L/min to maintain anesthesia. At the end of the procedure, the concentration of isoflurane was reduced gradually until the animal was on 100% oxygen and then gradually was changed to room air.

A modified crossover design with a minimum 1-week washout between anesthetic regimens was used (Table 1). A fourth anesthetic regimen, isoflurane inhalation by facemask only (Iso), was initially evaluated. The anesthetic machine, isoflurane vaporizer, and method of isoflurane administration were the same as above except a small to medium large animal mask with a dam that fit snugly over the snout was used, and no preanesthetic was administered. After the first week, further experimentation with Iso only inhalation was discontinued. The time necessary to induce anesthesia with Iso only and evaluate the sites using the bioengineering methods was consistently

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Table 1. Crossover design for anesthetic regimens.[a]

Animal	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
1	T/X	NA	T/X/Iso	NA	K/X	NA	NA	NA	NA
2	T/X	NA	T/X/Iso	NA	K/X	NA	NA	NA	NA
3	NA	T/X/Iso	NA	T/X	NA	NA	K/X	NA	NA
4	NA	T/X/Iso	NA	T/X	NA	NA	K/X	NA	NA
5	NA	Iso[b]	NA	T/X/Iso	NA	NA	T/X	NA	K/X
6	NA	Iso[b]	NA	T/X/Iso	NA	NA	T/X	NA	K/X

[a]T/X = tiletamine HCl/zolazepam HCl/xylazine HCl combination; T/X/Iso = tiletamine HCl/zolazepam HCl/xylazine HCl combination followed by isoflurane; K/X = ketamine HCl/xylazine HCl; Iso = isoflurane; NA = not applicable (washout period).
[b]Evaluation of Iso was stopped after the first week and replaced with K/X.

greater than the 2-hr time frame targeted. K/X was substituted, which compromised the crossover design. Animals were studied for 5–8 weeks, with K/X tested last for each animal. Unanticipated scheduling difficulties resulted in some variability in the length of the washout periods (1–2 weeks).

Each regimen (K/X, T/X, and T/X/Iso) was administered in three iterations within a week (every other day), with a minimum 1-week washout period before the next anesthetic regimen was tested. On each day of testing, each of the six experimental sites was evaluated in four successive testing rounds using three noninvasive bioengineering methods. All bioengineering methods were conducted in the same environmentally controlled room under similar conditions of lighting, temperature, humidity, and airflow. During each evaluation period, the methods were used in the following order: RC, TEWL, and LDPI. Site evaluations generally began 5–10 min after anesthesia was administered. Evaluations were made approximately in the center of each experimental site and were completed within a 2-hr period. When an animal showed signs of recovery (e.g., movement of head or extremities), additional anesthetic was administered. Dosing intervals thus varied among animals. The room temperature and humidity were measured within a tent as described below.

Bioengineering Methods

Reflectance colorimetry was used to measure red/green balance (a* coordinate of the three-dimensional color system recommended by the Commission Internationale de I'Eclairage in 1976). A Minolta Chroma Meter Model CR-300 (Minolta Corporation, Ramsey, NJ) was used. During each testing round, four replicate readings were taken and averaged from each of the six experimental sites to yield one measurement per site.

Stratum corneum barrier function was tested by measuring TEWL based on the vapor pressure gradient estimation method first described by Nilsson (19). An Evaporimeter Model EP-2 (Servo Med AB, Kina, Sweden) was used. All measurements were conducted under a protective three-sided tent (constructed from $^1/_4$ in. PVC piping and heavy mil plastic) to minimize the effect of air currents in the laboratory. A single measurement was taken from each experimental site over an approximate 1 cm^2 area during each of the four rounds using a single probe. Using Servo Med software, each

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measurement was conducted over 60 sec, with the instrument recording TEWL values five times per second. During the first 30 sec, the measurements climbed to a plateau. The 150 data points collected during the final 30 sec (at plateau level) were averaged, and a single TEWL measurement was recorded.

Laser Doppler perfusion imaging was used to study cutaneous blood flow (microcirculation), using a moorLDI™ (Moor Instruments, Inc., Wilmington, DE). A single measurement was taken from each experimental site during each of the four rounds. Operating parameters on the moorLDI were as follows: DC gain = 0, flux gain = 0, conc gain = 2 (gains were set against normal abdominal swine skin), background threshold = 200, distance = 20 cm, scan size = normal, scan speed = 10 ms/pixel, DC image resolution = 256 × 256 pixels, blood flux units (arbitrary units)

Table 2. Mean responses for each anesthesia regimen by anesthesia, iteration, and testing round.

			Mean Response (SE)			
Method	Anesthesia[a]	Iteration	Round 1	Round 2	Round 3	Round 4
RC[b]	K/X	1	8.01 (0.37)	7.56 (0.37)	7.36 (0.37)	7.38 (0.37)
		2	8.54 (0.38)	8.32 (0.38)	8.16 (0.38)	8.37 (0.38)
		3	8.14 (0.38)	7.61 (0.38)	7.38 (0.38)	7.44 (0.38)
	T/X	1	9.77 (0.37)	9.32 (0.37)	9.11 (0.37)	9.14 (0.37)
		2	9.47 (0.37)	9.25 (0.37)	9.10 (0.37)	9.30 (0.37)
		3	9.34 (0.37)	8.80 (0.37)	8.58 (0.37)	8.64 (0.37)
	T/X/Iso	1	9.44 (0.37)	8.99 (0.37)	8.79 (0.37)	8.81 (0.37)
		2	8.53 (0.37)	8.31 (0.37)	8.16 (0.37)	8.36 (0.37)
		3	10.24 (0.37)	9.70 (0.37)	9.48 (0.37)	9.54 (0.37)
TEWL[c]	K/X	1	11.46 (0.46)	10.59 (0.46)	10.59 (0.46)	10.01 (0.46)
		2	9.61 (0.46)	9.05 (0.46)	8.82 (0.46)	8.45 (0.46)
		3	8.58 (0.46)	7.82 (0.46)	7.73 (0.46)	7.71 (0.46)
	T/X	1	13.18 (0.46)	12.32 (0.46)	12.31 (0.46)	11.74 (0.46)
		2	11.33 (0.46)	10.77 (0.46)	10.54 (0.46)	10.17 (0.46)
		3	10.30 (0.46)	9.54 (0.46)	9.46 (0.46)	9.43 (0.46)
	T/X/Iso	1	13.49 (0.46)	12.62 (0.46)	12.62 (0.46)	12.04 (0.46)
		2	11.63 (0.46)	11.08 (0.46)	10.85 (0.46)	10.48 (0.46)
		3	10.60 (0.46)	9.85 (0.46)	9.76 (0.46)	9.73 (0.46)
LDPI[d]	K/X	1	698.55 (32.72)	637.89 (32.72)	642.22 (32.72)	655.58 (32.76)
		2	618.27 (32.71)	618.49 (32.71)	602.21 (32.71)	607.47 (32.71)
		3	570.57 (32.76)	570.29 (32.72)	566.04 (32.72)	570.12 (32.72)
	T/X	1	661.46 (32.71)	600.81 (32.71)	605.13 (32.71)	618.49 (32.72)
		2	586.90 (32.71)	587.11 (32.71)	570.83 (32.71)	576.09 (32.71)
		3	564.95 (32.72)	564.67 (32.71)	560.43 (32.71)	564.51 (32.71)
	T/X/Iso	1	540.73 (32.71)	480.07 (32.71)	484.40 (32.71)	497.76 (32.72)
		2	563.49 (32.71)	563.71 (32.71)	547.42 (32.71)	552.68 (32.71)
		3	512.09 (32.72)	511.81 (32.71)	507.56 (32.71)	511.64 (32.71)

[a]T/X = tiletamine HCl/zolazepam HCl/xylazine HCl combination; T/X/Iso = tiletamine HCl/zolazepam HCl/xylazine HCl combination followed by isoflurane; K/X = ketamine HCl/xylazine HCl.
[b]RC = reflectance colorimetry (a^*).
[c]TEWL = transepidermal water loss (g/m^2hr).
[d]LDPI = laser Doppler perfusion imaging (AU).

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set to "perfusion." All experimental sites were separately scanned with a centrally located scan area of 1.4 × 1.4 cm (70 × 70 pixel resolution). A defined region of interest (ROI) measuring 1.87 cm^2 was placed in the center of each recorded scan, and blood flux levels at each of the pixel points within the ROI were measured by using built-in image analysis software. The values at each of the pixel points were averaged, generating a single flux value for each site.

Statistical Analyses

Histograms of each of the response variables revealed that the distribution of each was bell-shaped and adequately approximated by a normal distribution. Once this determination was made, statistical models were fit to each of the responses. To determine the anesthetic regimen with the least effect on a response variable, mixed analysis of variance (ANOVA) models (with both fixed and random effects) were fitted separately to the RC, TEWL, and LDPI data, using the MIXED procedure in SAS® (V8). Only main effects and interactions that were statistically significant were included in the models. Statistical significance was defined as $p < 0.05$. Several main effects were of interest: anesthetic regimen, sampling iteration, ventral site on animal, and round within sampling iteration. An interaction between anesthesia and sampling iteration was present for both RC and LDPI responses. The random effect was the animal. An interaction between sampling iteration and animal was present for all responses and was included as an additional random effect. To determine the anesthesia with the least effect, statistical contrasts were used to determine whether there were significant differences among mean response levels for the three anesthesia regimens, while controlling for other factors in the model. Model estimated means of the RC, TEWL, and LDPI data for each anesthesia regimen, iteration, and round of testing are presented in Table 2 for the assessment of changes over time. In addition, model estimated means of RC, TEWL, and LDPI response over all test iterations and rounds are presented in Table 3 for each anesthesia regimen. The overall means provide a broad comparison of the anesthesia regimens.

RESULTS

The average room temperature during the experiment was 23.7°C ± 0.67 standard deviation (SD), and the average humidity was 38.2% ± 3.0 SD. Animals appeared healthy during the course of the experiment, with an average body temperature of 39.0°C ± 0.8 SD.

Iso used on the first two animals during the first week of the study required 45 min of manual restraint (anesthetic inhalation mask) to induce adequate anesthesia for allowing bioengineering assessments on an animal in dorsal recumbency. The assessments required a minimum of 90 min to conduct. Because the total length of those experiments exceeded a 2-hr time limit, this regimen was abandoned after the first two animals and replaced by K/X. One injection of K/X or T/X was sufficient to put the animals into a plane of anesthesia sufficient for dorsal recumbency and assessments. Additional injections were needed to complete the readings within the 2-hr time limit. K/X required a total of 6–10 i.m. injections, and T/X needed 2–4 i.m.

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Table 3. Mean responses for each anesthesia regimen, over all test iterations and rounds.

Bioengineering method	Regimen T/X: Mean (SE)	Regimen T/X/Iso: Mean (SE)	Regimen K/X: Mean (SE)	Difference in response (p-value)		
				T/X-T/X/Iso	T/X-K/X	T/X/Iso-K/X
RC[a]	9.15 (0.30)	9.03 (0.30)	7.86 (0.30)	0.12 (0.214)	1.30 (< .001)	1.17 (< .001)
TEWL[b]	10.92 (0.32)	11.23 (0.32)	9.20 (0.32)	−0.31 (0.038)	1.72 (< .001)	2.03 (< .001)
LDPI[c]	588.45 (22.86)	522.78 (22.86)	613.14 (22.87)	65.67 (< .001)	− 24.69 (0.007)	− 90.36 (< .001)

[a]RC = reflectance colorimetry (a^*).
[b]TEWL = transepidermal water loss (g/m^2hr).
[c]LDPI = laser Doppler perfusion imaging (AU).

injections. T/X/Iso required one injection of the tiletamine HCl/zolazepam HCl/ xylazine HCl combination followed by intubation and connection to the isoflurane vaporizer for each test. Usually, the animal's larynx was edematous after the second test day. Repeated intubations were difficult, and the animals had difficulty breathing after removing the endotracheal tube. In addition, the depth of anesthesia was not controlled well with this regimen.

The average response by anesthetic regimen, iteration, and testing round for RC (red/green balance) is presented in Table 2. For any given iteration, the first sampling round had the highest response for all three regimens, whereas later rounds had lower responses. Regimens T/X and K/X had a narrower range of readings over the 3 testing days than T/X/Iso. Overall, K/X yielded the lowest response and was statistically different from T/X and T/X/Iso, which gave higher responses that were not significantly different from each other on average (Table 3).

The average response by anesthetic regimen, iteration, and testing round for TEWL is presented in Table 2. For any given iteration, the first sampling round had the highest response, and the fourth sampling round had the lowest response. Mean responses declined over 3 days of experiments for each regimen. Overall, K/X yielded the lowest response, statistically different from T/X and T/X/Iso. Regimens T/X and T/X/Iso yielded higher responses that were not significantly different from each other on average (Table 3).

The average response by anesthetic regimen, iteration, and testing round for LDPI can be seen in Table 2. The three regimens behaved similarly within the same day: the first sampling round had the highest response for each sampling iteration, with the readings generally decreasing over the first three rounds and increasing slightly for the fourth round. Mean response declined on later study days for T/X and K/X, but not for T/X/Iso. Overall, K/X yielded the highest response and T/X/Iso the lowest. Mean responses were significantly different among all three regimens (Table 3).

The same-day data indicated that the readings for each bioengineering method were affected over time for each anesthetic regimen.

DISCUSSION

We routinely use a reflectance colorimeter to assess erythema (a*) response following SM exposure (10,11,20,21). For a general overview of RC, the reader is directed to articles by Elsner (22) and Westerhof (23). In this study, T/X and T/X/Iso yielded a higher RC response than did K/X, indicating a greater blanching effect for K/X. The RC was sensitive to the time following induction of anesthesia. Regimens T/X and K/X had a narrower range of readings over the 3 testing days than T/X/Iso. Thus, either T/X or K/X was suitable, with T/X preferred due to a lesser blanching effect.

Evaporimetry has been used to test cutaneous irritants, monitor epidermal repair processes in burns, and evaluate clinical skin conditions (irritations or diseases) and occlusive pharmaceutical/cosmetic preparations (24). For a general overview of TEWL, the reader is directed to Pinnagoda et al. (24,25) and Elsner et al. (26). Recent SM-induced burn studies suggest that evaporimetry is a viable, noninvasive method to determine when normal barrier function is compromised and/or returns to normal. SM wound healing studies indicate that evaporimetry measurements to assess barrier

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function is the best indicator of healed wounds (10). In this study, there was a decline in TEWL response over time within the experimental days that indicated that TEWL was sensitive to the time following induction of anesthesia. A decreased skin temperature as a result of decreased blood perfusion (27) likely played a role in the TEWL declines. Skin surface temperature is one of the essential factors that dictate the rate of TEWL in normal skin (24,25,28–30). In normal human skin, TEWL has been shown to increase exponentially with skin temperature both ex vivo (31,32) and in vivo (25,30,33). A similar finding has been noted in hairless mouse skin; however, the effect is more pronounced in this species than that found in man (34). The positive relationship of TEWL to skin temperature in psoriasis and eczema is different from that obtained in normal skin and can be explained by both exponential and linear models (35). Mathias, Wilson, and Maibach have developed an equation that can be used to convert TEWL at any given skin surface temperature of human skin to a common reference temperature of 30°C, to facilitate inter- and intrasubject comparisons (31). Fluid loss during anesthesia may have also played a role in the decline of TEWL response over time, as a result of shifts between fluid compartments and/or slight diuretic effects. In this experiment, no intravenous fluids were administered. Fluid administration during anesthesia may have compensated for any effect on TEWL due to fluid loss. Further experimentation is necessary to elucidate the relative effects of blood flow, skin temperature, and fluid loss on TEWL rates in this animal model. In this experiment, TEWL response also declined over multiple treatment days. Repeated administration of anesthesia every other day may not allow sufficient time for normal levels of skin hydration to be restored. T/X or T/X/Iso were preferred for TEWL readings, because overall they appeared to depress TEWL rates to a lesser extent than did K/X. Although statistically significant differences were noted, they may have held no biological significance (e.g., they may not have represented any major functional difference based upon the relatively low TEWL rates noted).

Laser Doppler flowmetry and LDPI are widely used for prolonged, noninvasive monitoring of tissue viability and wound healing. In addition, these techniques are used to assess peripheral vascular disease, inflammation, ischemia, reperfusion, skin graft acceptance, and burn depth (10). General overviews of these techniques are available (36–41). In studies involving SM and Lewisite vapors, Chilcott et al. (42) concluded that although RC and TEWL measurements could provide quantitative, noninvasive methods for determining efficacy of candidate treatment regimens, neither is comparable to the prognostic capabilities of LDPI. Although T/X, T/X/Iso, and K/X yielded significantly different blood flux response levels in this study, none was clearly preferable, because all three affected cutaneous blood flow. The more anesthesia the animal was given during the 5-day measurement period, the greater the reduction in flow. The reduction in cutaneous blood flow over multiple treatment days was particularly evident with the injectable anesthetics, and was probably the result of residual anesthetic in the body. Inhalation anesthetics, such as isoflurane, are eliminated from the body more completely and in less time than injectable anesthetics (17). Inhalation anesthetics are preferable where residual anesthetic affects may be a concern.

A self-imposed 2-hr time constraint for anesthesia induction and bioengineering assessment was exceeded when using Iso. Forty-five minutes of manual animal restraint were required to administer isoflurane by mask to the desired level before assessments began. This excessive amount of time was due primarily to the animals struggling

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while being manually restrained, thereby preventing a perfect seal between the mask and the snout. Although restraint systems such as the Panepinto Sling™ would have aided in this endeavor, they cannot be easily used with an animal model that incorporates lesions on the abdominal skin without interfering with the integrity of those lesions. This regimen was discontinued after the first week of testing in two animals. A different method of manual restraint, training animals to accept the mask, using a whole-body exposure chamber rather than a mask, or increasing the gas flow rate with a higher concentration of isoflurane may minimize the length of time needed to reach adequate anesthesia. Further testing with Iso under alternate animal handling and anesthetic protocols may reveal that this anesthetic regimen can provide consistent and minimal effects on the noninvasive bioengineering methods tested.

CONCLUSIONS

Overall, T/X (repeated i.m. injections of the tiletamine HCl/zolazepam HCl/xylazine HCl combination) is the anesthetic regimen recommended for bioengineering assessments (RC, TEWL, and LDPI) of ventral abdominal skin in swine. T/X produced the most consistent results with fewer anesthetic effects than the other regimens. Relating bioengineering parameters measured within skin lesions to data collected from control sites or normal perilesional skin will help minimize the effects of anesthesia (10).

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